Building a Virtual Model of a Baleen Whale: Phase 2

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LONG-TERM GOALS

This project proposes to CT scan an entire baleen whale and, eventually, build a vibroacoustic model that will allow us to simulate how sound interacts with the whale's anatomy.

OBJECTIVES

The project has been subdivided into three phases. Phase 1 has been completed; we constructed the overall strategy and constructed a specialized bag that will be used to tow a dead whale to a boat haul-out facility. This project covers Phase 2, where the objectives are primarily technological. We will construct more basic equipment, conduct some tests, and prepare for an attempt to capture a whale carcass. The basic plan is to capture a postmortem California Gray Whale (*Eschrichtius robustus*) (Lilljeborg, 1861) after it dies along the annual migration route. The specimen will then be cooled, towed to a haul-out yard, placed in a specially designed container, and then transported to a commercial freezer. Eventually, the specimen will be transported to an industrial sized CT scanner. After the CT scans are conducted on the entire specimen the whale will be transported to the Smithsonian Institution where it will be taken apart so that the tissue properties can be measured. The CT scan data and tissue property measurements will be used to construct a finite element model in Phase 3, planned for a future proposal.

APPROACH

Key Personnel

- **Captain Jim Christmann**, RV Shana Rae and Monterey Canyon Research Vessels, Inc., Santa Cruz, CA.
- **Mr. David Jablonski,** Sanctuary Stainless, Moss Landing California.
- **Dr. David Casper (DVM),** Director, Long Marine Lab Marine Mammal Stranding Network, **University of California, Santa Cruz.**
- **Professor Petr Krysl,** Department of Engineering, University of California, San Diego.

Report Documentation Page

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Justification

Oceanic sound levels, especially low-frequencies from geologic exploration, industrial development, shipping, and military activities, have increased steadily over the last half-century (McCarthy, 2005). Low frequency sounds have been known to negatively impact large whales (Frantzis, 1998; Balcomb and Claridge, 2001; Malakoff, 2002) and possibly other living marine resources.

Navy sonar training operations have been hampered by concerns and lawsuits over the effects that high intensity sound exposure might have on marine organisms, specifically the mammals. Since the Navy has been tasked to understand any impact that its operations might have on living marine resources, it is important to work toward a methodology that will provide facts that will promote the assessment of vibroacoustic impact.

There is worldwide interest in the potential effects of anthropogenic sound on mysticete (baleen) whales. Most of the research on the effects of sound has been conducted on a few small marine mammal species that can be housed in research labs and aquaria but little is known about large marine mammals, like mysticetes. Long wavelength, low-frequency sounds are likely to have their most significant interaction with the bodies of these large animals. The large size of these animals precludes the potential to work with them in captivity in any meaningful way. Consequently, the most effective way to study the vibroacoustic physiology of these animals is to build a model of mysticete anatomy to study the interaction between these animals and low-frequency sound. Improvements in industrial-grade x-ray computed tomography (CT) scanners have made it feasible to scan an adult mysticete.

Modeling has several advantages. Models are flexible with respect to species and the variety of acoustic stimuli that can be tested. Once developed, models are also inexpensive to reuse in light of new information or apply to new questions. The models we propose to build are constructed at the organism level. This allows us to investigate interactions on the whole organism or to zoom in on structures or suites of structures to address questions of sound propagation and transmission across interfaces, distribution of acoustic pressure and shear stresses, dissipated energy and heating effects, excessive strains or displacements due to resonance, potential to induce cavitation, and produce synthetic audiograms.

Our team has pioneered a suite of techniques that combine the anatomic geometry obtained from CT scans (Cranford, 1988; Cranford *et al.*, 1996; Cranford *et al.*, **2014**) with measurements of tissue elasticity (Soldevilla *et al.*, 2005; Hess *et al.*, 2006) and custom FEM software (Krysl *et al.*, 2006), the *vibroacoustic toolkit* (VTk). This combination produces a versatile computational environment for vibroacoustic simulations (Krysl *et al.*, 2008). This suite of techniques can also be used to assess acoustic exposure across a broad taxonomic spectrum.

The intellectual merit of these methods has been demonstrated by the recently published discovery of a new pathway for sound entering the head of a beaked whale (Cranford *et al.*, 2008a), a result that challenges the long accepted paradigm of toothed whale hearing (Norris, 1968). In addition, anatomic similarities with all living toothed whales suggest that this new pathway may also be the original pathway used by the ancient whales (archaeocetes) in the Eocene. This discovery was catalyzed by the disparate views and collective efforts of experts in different disciplines, the essence of our approach.

These computer-enabled investigative methods have already transformed our capacity to generate original knowledge and understand the bioacoustics of marine mammals (Cranford, 2000; Cranford

and Amundin, 2003; Cranford *et al.*, 2008a; Cranford *et al.*, 2008b; Cranford *et al.*, **2014;** Lancaster *et al.*, 2014). The resulting simulations allow us to emulate, for example, the sound generation mechanism and the formation of an acoustic transmission beam, or to measure the amplitude differences and time delays for sounds reaching each of the ear complexes, or produce synthetic audiograms for species that are otherwise inaccessible for study. These are just a few examples of the predictions and understanding we can glean from basic vibroacoustic simulations, *all of them from inside the organisms*.

WORK COMPLETED

1. Designed, purchased, and tested recirculating seawater refrigeration system. We tested the effectiveness of our ability to cool a mass of seawater contained in the towing bag while the ship and apparatus were moored at the MBARI facility in Moss Landing, CA. The tests showed that more insulation was required in the wall of the bag. Consequently, we redesigned the bag to hold strips of neoprene and sent it back to Seattle to add the new construction. We also modified the net reel to accept the added volume of the modified bag. A new thermal test has been scheduled for January of 2014. This is part of the effort to acquire and scan a complete **sub-adult or adult gray whale** (*Eschrichtius robustus*) carcass.

2. We acquired the head of a freshly postmortem **neonate fin whale** (*Balaenoptera physalus*) and conducted a high resolution CT scan of the entire head using an industrial CT scanner at Hill Air Force Base in Utah. The anatomic components have now been segmented (see images below). The process of meshing the anatomic components to build a vibroacoustic model is complete. We have generated preliminary results that demonstrate the sound reception mechanisms and frequency sensitivity from synthetic audiograms (see **RESULTS** section).

3. We acquired a complete **juvenile minke whale** (*Balaenoptera acutorostrata*) carcass and prepared the entire carcass for CT scanning at Hill Air Force Base in Utah. The specimen stranded alive and was in good condition when it died. Scanning was delayed but is scheduled to begin in March 2014.

4. In a collaborative effort, we acquired an ear block from a **neonate gray whale** (*Eschrichtius robustus*) and have conducted high resolution CT scans at the Digimorph facility at University of Texas, Austin. Segmentation of this specimen is underway.

5. We acquired an ear block from an **adult sei whale** (*Balaenoptera borealis*), conducted a CT scan and segmented the structures.

It should be clear from the list of work completed that we have accomplished broad coverage of mysticete species and sizes. This will be extremely valuable for the comparative perspective in the vibroacoustic analysis for the project. In fact, we are already slightly ahead of schedule since the initial stages of modeling are complete and we have already begun to produce preliminary results. For example, we have generated synthetic audiograms for a fin whale calf.

RESULTS

Phase 2 of the project has now sprouted four new tracks that are running simultaneously and in parallel with the original track. These five tracks can be succintctly stated as follows:

(1) Gray whale (*Eschrichtius robustus*) (Lilljeborg, 1861). The original first track as in the proposal, planning for aquisition of an entire postmortem gray whale from along the coastal California migration routes to be CT scanned.

(2) Fin whale (*Balaenoptera physalus*) (Linnaeus, 1758). Segmentation of CT scans from the intact head of a neonate fin whale (*Balaenoptera physalus*). Preliminary results for sound reception mechanisms and frequency sensitivity using finite element modeling tools to generate synthetic audiograms.

(3) Common minke whale (*Balaenoptera acutorostrata*) (Lacépède, 1804**).** Aquisition, preparation, and CT scanning of an entire juvenile minke whale.

(4) Ear of Gray whale (*Eschrichtius robustus*) (Lilljeborg, 1861). Aquisition of a neonate gray whale (*Eschrichtius robustus*), extraction of the intact ear complex and high-resolution CT scanning is complete, segmentation currently underway, eventually leading to vibrational analysis. .

(5) Sei whale (*Balaenoptera borealis*) (Lesson, 1828). Aquisition, CT scanning, and segmentation of an ear block containing and ear complex from an adult sei whale.

The following paragraphs describe some details of current results for each of the five tracks.

(1) Gray whale (*Eschrichtius robustus*) (Lilljeborg, 1861).The original first track.
Collaborators: Capt. Jim Christmann and Dave Jablonski.

Figure 1.1 - The container tow bag was filled with water in the harbor while moored at the MBARI facility in Moss Landing, CA, California, where the thermal conductance tests were performed.

Figure 1.2 - The refrigeration unit on the deck of the R/V Shana Rae was used to chill the water recirculating in the container bag. The test did not acheive a sufficient rate of reducing the water temperature and it was determined that the bag needed additional insulation. Consequently, we designed a series of elongate parallel pockets that were then built into the wall of the bag, in which we inserted strips of 1/2 inch neoprene. Planning is underway for another thermal test in March 2014.

(2) Fin whale (*Balaenoptera physalus*) (Linnaeus, 1758). Segmentation of CT scans from the intact head of a neonate Fin Whale (*Balaenoptera physalus*).
Collaborators: Judy St. Leger, Petr Krysl, Jennifer Jeffress.

This neonate female fin whale head is 131 cm long and 73 cm wide. It has been CT scanned and segmented in preparation for simulating sound reception using our Vibroacoustic Toolkit (VTk). These are the first simulations of sound recpetion mechanisms for an entire mysticete head (see preliminary results below).

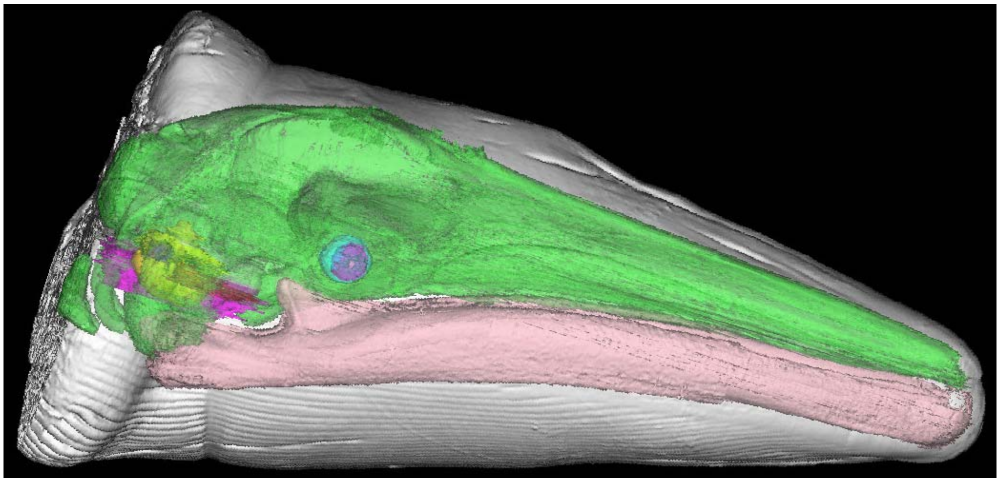

Figure 2.1 - Lateral view of the head of a fin whale neonate: Skin=white; skull=green; mandibles=pink; TPCs=yellow; peribullary sinuses=magenta. The eye on the side of the head is shown as a reference point.

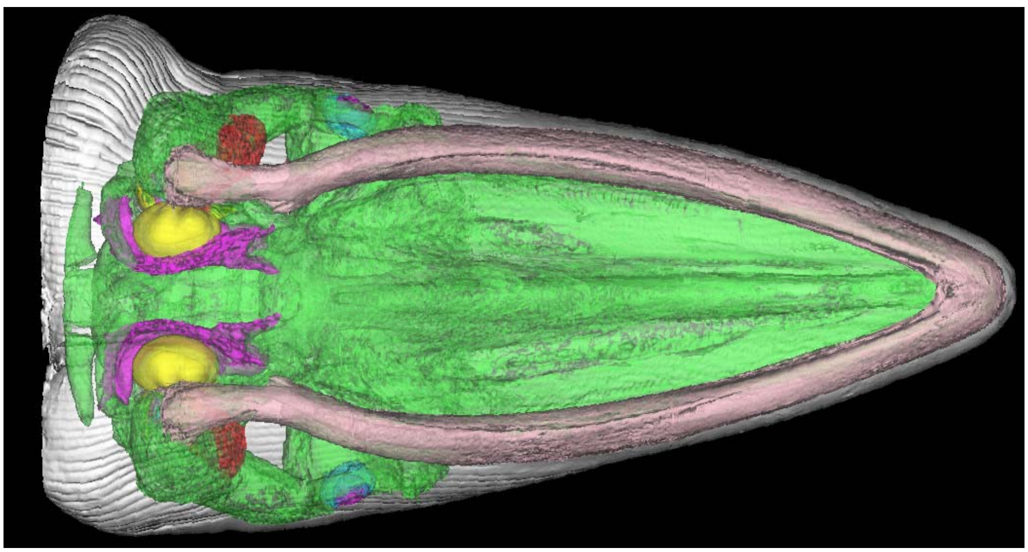

Figure 2.2 - Ventral view of the head of a fin whale neonate: Skin=white; skull=green; mandibles=pink; TPCs=yellow; peribullary sinuses=magenta; low density tissue channels=red. The eyes on the sides of the head are shown as reference points.

Pressure Loading and Bone Conduction Mechanisms in Mysticete Sound Reception

We have generated synthetic audiograms for a fin whale using finite element modeling simulations and CT scans from a *Balaenoptera physalus* calf.

Our simulations show that there are two mechanisms that excite the tympanoperiotic complex (TPC) in mysticetes, the ***pressure-loaded mechanism*** and the ***bone conduction mechanism***. One of these mechanisms is more dominant than the other. In the *less* dominant ***pressure-loaded mechanism***, acoustic pressure waves reach the TPC through the seawater and soft tissue pathways, resulting in direct pressure loading on the tympanic bulla. The dominant ***bone conduction mechanism*** is characterized by deformation of mysticete skull bones when the acoustic pressure waves scattering off

of them. During ***bone conduction***, excitation of the hearing apparatus results from the difference in the inertial properties between the skull bones and the TPC.

This bold statement about a ***bone conduction*** mechanism is also supported by the structure of the tympanoperiotic complex (TPC) in mysticetes. **Figure 2.3** shows that the periotic portion of the TPC in mysticetes is rigidly anchored to the skull by multiple dense bony processes or "projections" into the basicranium (Ekdale *et al*., 2011). The posterior process of the periotic is wedged between the squamosal and the exoccipital, while the anterior process projects between the squamosal and the pterygoid bones of the skull (Dwight, 1872).

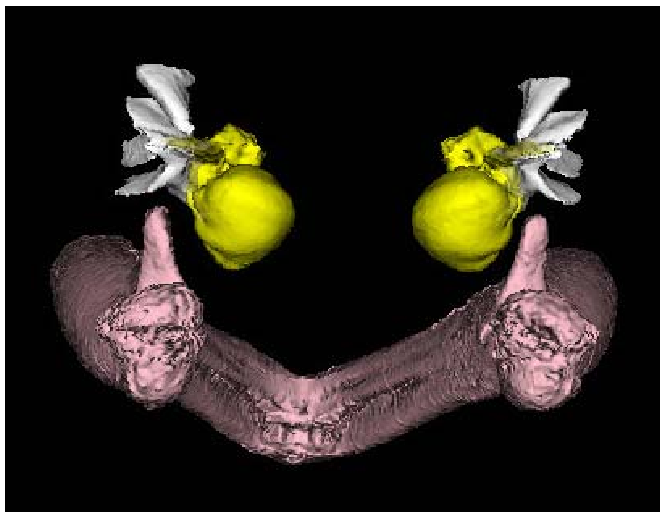

Figure 2.3 - Posterior view of both TPC's (yellow) with the bony processes (white) that anchor each TPC rigidly to the skull of the fin whale (*Balaenoptera physalus*). Mandibles (pink) are shown for context.

Another important structural feature that is essential to the mysticete ***bone conduction mechanism*** is the extreme density difference between the TPC and the other bones of the skull. This is nicely summarized in the following quote by Yamada (1953):

> "In general, I cannot emphasize too much that the cetacean tympanoperiotic bone is extraordinarily so compact and so dense like the enamel substance of the mammalian teeth that the bone can be sawed with great difficult and fortitude; whereas other bone are mostly spongy and impregnated with much oil."

The disparate density values between the TPC and most of the other skull bones, translate into inertial differences. These inertial differences contribute to the sound reception process by producing differences in the vibrational characteristics of these components.

Two finite element models were required to reveal each sound reception mechanism. The two models calculate different portions of a single simulation, whose results must be combined to reveal the complete mechanism, ***pressure-loaded*** or ***bone conduction***.

The first model broadcasts an acoustic pressure wave through the water toward the head from in front of the animal. The sound waves travel through seawater, reach the head, continue along soft tissue pathways (that have acoustic impedance values similar to water), and impinge upon the skull and the

tympanic bone or "bulla" of the TPC. The second model simulates what happens at the tympanic bulla.

In the ***pressure-loaded mechanism*** forces are exerted upon the tympanic bulla by the sound pressure waves that travel through water and soft tissue. The extreme impedance mismatch between soft tissue and the dense tympanic bone causes the maximum force from the acoustic pressure waves to be exerted upon the tympanic bulla at the interface with soft tissue (Cranford *et al.*, 2010). Since the malleus is fused to the bulla, the force applied to the tympanic bulla will cause some motion in the ossicular chain, resulting in motion at the stapes footplate, which sits in the oval window of the cochlea. The pressure delivered to the surface of the tympanic bulla is the source of forcing for the ***pressure-loaded mechanism***.

The two models that predict the ***pressure-loaded mechanism*** are essentially identical to those that we used to investigate sound reception in toothed whales (Krysl *et al.*, 2006; Cranford *et al.*, 2010; Cranford and Krysl, 2012) and led us to the understanding that the odontocete head works like an acoustic antenna. This notion of the head-as-an-acoustic-antenna probably applies to mysticetes too.

The ***bone conduction mechanism*** was revealed by using the same two models; one model follows the paths of the acoustic pressure waves that interact with the head, and the other model tracks the response of bony TPC. In the ***bone conduction mechanism***, the combined results of the two models describe the scattering interaction between the acoustic pressure waves and the skull. As is the case with mysticete sound reception, some of the acoustic wavelengths are much longer than the skull, so for low frequencies it can be further classified as Rayleigh scattering (Rayleigh, 1896). As a result of scattering the sound waves, the skull deforms and moves in response to the passage of the pressure waves.

At the same time, the tympanic bullae are dense appendages attached to the periotic portion of the TPC through two thin, relatively flexible pedicles that are forced to follow the vibrations of the skull. Each tympanic bulla forms a lever arm between the fulcrum at the pedicles and the large bony mass at the distal end (**Figure 2.4**), a structure known as the involucrum (Mead and Fordyce, 2009). The lever arm and inertial properties of the involucrum cause differential motion between the bulla and the related skull bones, which in turn cause motion in the ossicular chain, since the malleus is fused to the tympanic bulla.

The second model incorporates the forcing, through the motion of the skull, by prescribed harmonic-motion of the periotic bones. This ***bone conduction mechanism*** appears to yield considerably more sensitivity, particularly at low frequencies, than the ***pressure-loaded mechanism***.

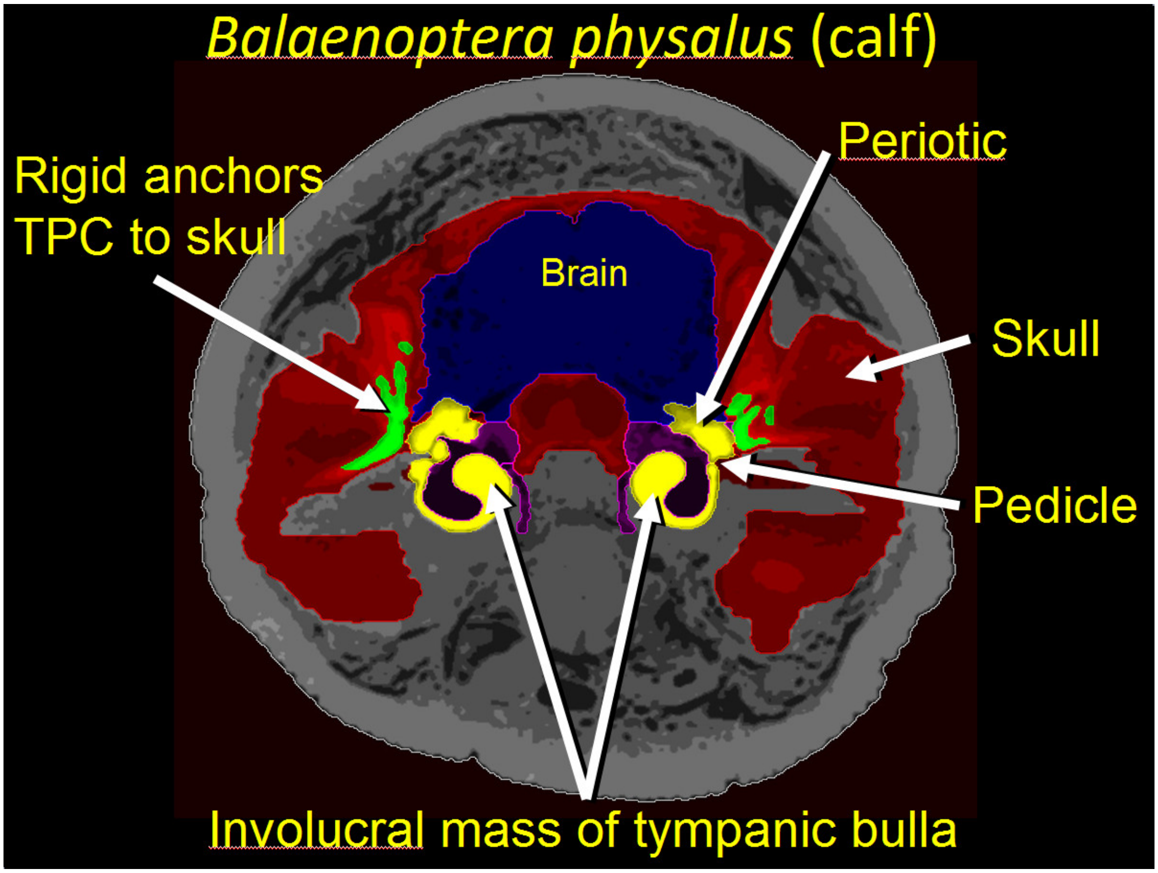

Figure 2.4 - Transverse section through the head of a fin whale calf. The bony projections that anchor the tympanoperiotic complexes to the skull are green. The brain is blue and the skull is red. The tympanoperiotic complexes are yellow. Note the thin bony pedicles that form a fulcrum, opposite the large dense masses at the distal end of the involucrum.

Preliminary analysis indicates that the ***bone conduction*** mechanism is largely responsible for the mysticete whale's sensitivity to low-frequency sound. According to the synthetic audiograms generated by our finite element models (**Figure 2.5**), the ***bone conduction*** audiogram is approximately four times more sensitive (lower threshold) between 1-2 kHz than the ***pressure-loaded*** audiogram. The difference in sensitivity over the range of the lowest frequencies (10 Hz to 130 Hz), is between 10 to 50 dB more sensitive for the ***bone conduction*** mechanism than for the ***pressure-loaded*** mechanism. This is the first study to predict relative sensitivities for mysticete sound reception over this broad range between 10 Hz to 12 kHz. With few exceptions, the ***bone conduction mechanism*** produces the lowest thresholds across that entire range (**Figure 2.5**). The ***bone conduction mechanism*** may be the dominant component in mysticete hearing.

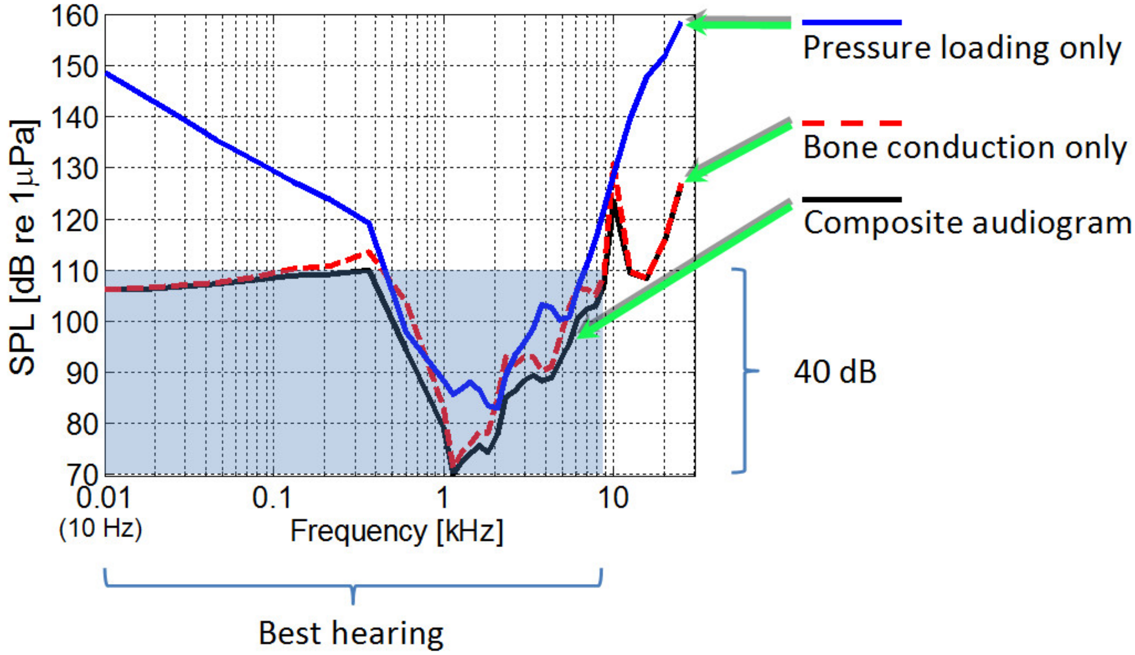

Figure 2.5 - Predicted audiograms for the fin whale calf. The blue line represents the audiogram for the ***pressure-loaded mechanism***. The red dashed line represents the audiogram for the ***bone conduction mechanism***. The black line shows the combined ***pressure-loaded*** and ***bone conduction*** audiograms.

Finally, any system of finite element models and their simulations requires at least one validation test, where the results of the simulations are compared to experimental results in the real word. Without validation the virtual models should be approached with cautious skepticism. We have published the first successful validation test of our modeling system, Cranford *et al*., (**2014**), and therefore have confidence in our simulation results.

(3) Common minke whale (*Balaenoptera acutorostrata*) (Lacépède, 1804)**.** Aquisition, preparation, and CT scanning of an entire sub-adult minke whale. The specimen was 366 cm long (12 ft).
Collaborators: Charley Potter, Maya Yamato, John Ososky, and James G. Mead.

Acknowledgements:
Joseph Robert Villari, MJ Adams, Steven Thornton, Donald E. Hurlbert, Sentiel (Butch) Rommel, Alexander M. Costidis, Stoyer, Richard, Brian Abrams, Ignacio Moreno, Jamie Testa, Brenda Kibler, Cindy Driscoll, Jennifer Dittmar, Brent Whitaker, Kerry Button, Kristofer Helgen, Nicholas Pyenson.

We acquired a minke whale that stranded alive on the Maryland coast. State veterinarians determined that the animal had to be euthanized. A crew from the Smithsonian Institution collected the specimen immediately upon death and transported it to a freezer at their facility in Suitland, MD.

Figure 3.1 - Arrival of entire minke whale specimen for processing. This specimen was very fresh.

Figure 3.2 - The minke whale was frozen and prepared for bisection and wrapping before positioning in the sarcophgus.

Figure 3.3 - Morphometric measurements were taken prior to sectioning.

Figure 3.4 - The intact head provided a basis for simulating sound reception using finite element modeling.

Figure 3.5 - Attaching plastic rods to sarcophagus casing. This allows for density calibration and alignment or "registration" of scans with one another.

Figure 3.6 - The outer wall of the sarcophagus with plastic rods attached for calibration during CT scanning.

Figure 3.7 - Anterior portion of the minke whale before wrapping and insertion into the custom built sarcophgus.

Figure 3.8 - Tail section of the minke whale wrapped for encasement in the specially designed sarcophagus.

Figure 3.9 - This image shows the first view of an entire minke whale packaged in the sarcophagus to be CT scanned. It will be scanned in January 2014, reconstructed volumetrically from the scans, segmented, and meshed for finite element modeling. This specimen will provide the first capability for simulating sound propagation into and out of the entire body of a mysticete whale. Our modeling process has recently been validated (Cranford *et al.*, **2014**).

By measuring a diverse sample of cetacean skulls and tympanoperiotic (ear) complexes, we have shown that precocial development of the bony ear complex is widespread and probably common to all cetaceans (Lancaster *et al.*, submitted). We have also provided evidence that our modeling

methodology is valid by comparing results gleaned from live animals involved in biosonar tasks (Cranford and Krysl, 2013) and have begun the process of understanding and describing how the cetacean head works like an acoutic antenna (Ary *et al.*, 2014; Krysl and Cranford, 2014), gathering inputs from many points on the skin and processing them according to frequency and input location.

(4) Gray whale (*Eschrichtius robustus*) ear (Lilljeborg, 1861). Aquisition of a neonate gray whale (*Eschrichtius robustus*), extraction of the intact ear complex and high-resolution CT scanning for eventual vibrational analysis.
Collaborators: Annalisa Berta, Eric Ekdale, Tom Demere, Maya Yamato.

This neonate gray whale specimen was collected by colleagues from San Diego State University and the San Diego Natural History Museum. They have kindly provided us with access to this specimen and the ability to conduct our vibrational analysis with fresh frozen tissue. This is in contrast to our previous work with odontocete TPC's that are normally fixed in formalin or cleaned and dried extensively for museum preparations. Muesum preparations of mystiete specimens are virtually always incomplete because the ossicles are normally loosely held in place and the incus falls out during preparation. The high-resolution scans have been completed and we are in the process of segmenting them. Vibrational analysis will follow.

Figure 4.1 - This neonate gray whale specimen has been acquired and dissected with colleagues at San Diego State University. We have extracted one of the ears and subjected it to high resolution CT scanning. The fetal hair follicles are indicated by the pins on the anterior aspect of the lower jaw.

Figure 4.2 - This is the ventral side of the neonate gray whale head before extraction of the ear complex.

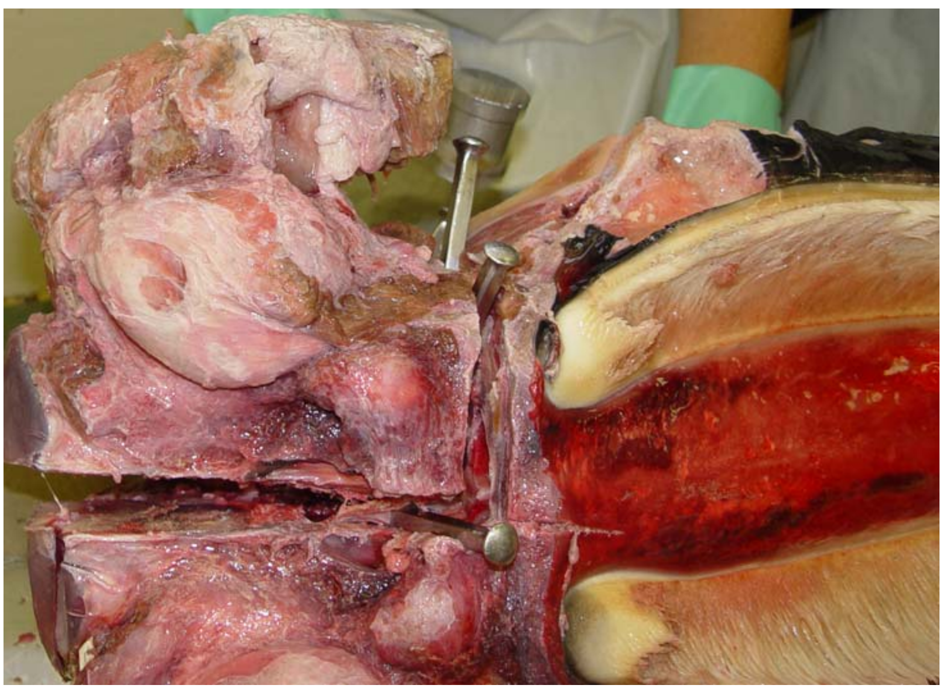

Figure 4.3 - This is the block of fresh frozen tissue that was extracted from the neonate gray whale. To our knowledge, this is the first extraction an a gray whale ear block for CT scan analysis.

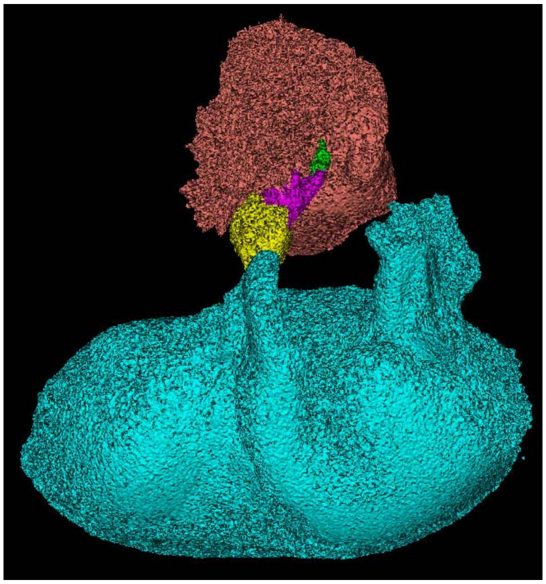

Figure 4.4 - Reconstruction of neonate gray whale TPC from high-resolution CT scan of the neonate gray whale ear. The tympanic bone is cyan, the malleus is yellow, the incus is magenta, the stapes is green, and the periotic is brown. The ossicles are arranged more linearly in mysticetes than in odontocetes.

(5) Sei whale (*Balaenoptera borealis*) (Lesson, 1828). Aquisition, CT scanning, and segmentation of an ear complex from an adult Sei whale.
Collaborators: Charley Potter, John Ososky, and James G. Mead

We acquired a preserved ear block of an ***adult*** sei whale from the Smithsonian instutition in Washington, D.C. The ear block has now been CT scanned and segmented. It is currently being prepared for vibrational analysis. This will allow us to perform the first vibrational analysis conducted on the ear of an adult mysticete.

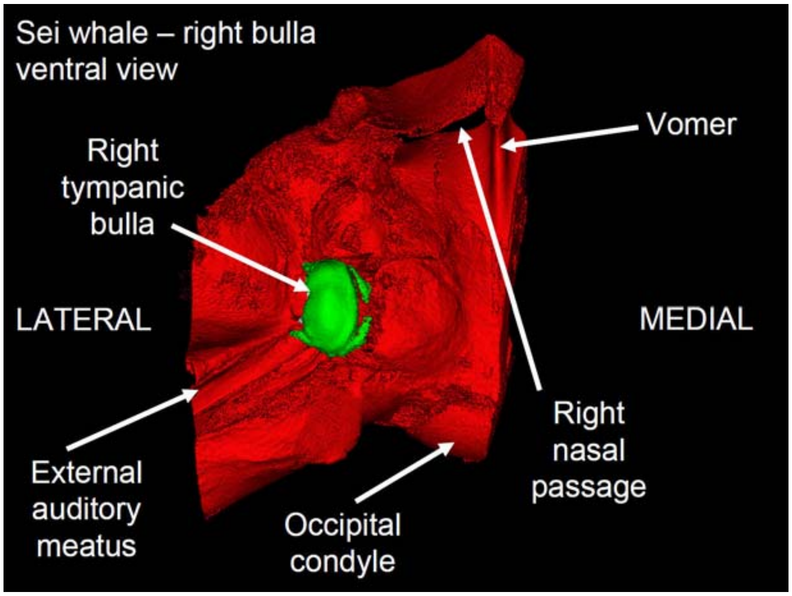

Figure 5.1 - Ventral view of the right tympanoperiotic complex, reconstructed from CT scans of an adult sei whale.

IMPACT/APPLICATIONS

Transitions and implications

The success of this project will mark a sudden and conspicuous transformation in our understanding of the anatomy and sound reception mechanisms in mysticetes. Clearly, the methodology developed for this project will greatly advance our understanding of the functional morphology of mysticete bioacoustics.

There are two major advancements that accrue from capturing in situ anatomy in an entire mysticete whale as a means for understanding acoustic function: the geometry of anatomy and an advantageous perspective. That is, the sizes, shapes and material composition of organs and tissue interfaces will determine their interaction with acoustic stimuli. In addition, it is very difficult to comprehend the anatomic structure of a mysticete by relying solely upon traditional methods (dissection) because the structures are much larger than the observer and any attempts to separate the slumping parts will all but destroy the indispensable anatomic geometry. We are still the only group of researchers who have been able to scan complete whale carcasses and it is currenlty the only technique that can provide anatomic geometry for any complete cetacean.

Combining CT scanning of large whales with computer modeling has several advantages. Models are flexible with respect to species and a wide variety of acoustic stimuli that can be tested. Once developed, models are inexpensive to reuse in light of new information or address to new questions. The models we build are *constructed at the organismal level*. This allows us to investigate interactions with respect to the whole organism or to zoom in on structures or suites of structures to interrogate questions of sound propagation and transmission across interfaces, distribution of acoustic pressure and shear stresses, dissipated energy and heating effects, excessive strains or displacements due to resonance, potential for cavitation, and any other mechanical impact.

The long-term, overarching research effort put forth here is robust and can inform regulatory decisions about the effects of these sounds on large marine mammals and fish.

A critique of Tubelli et al. (2012)

Recently a group of our colleagues published a paper that purports to predict the middle ear transfer function (METF) in a minke whale [Tubelli, A.A., Zosuls, A., Ketten, D.R., Yamato, M., Mountain, D.C. 2012. A prediction of the minke whale (*Balaenoptera acutorostrata*) middle-ear transfer function. The Journal of the Acoustical Society of America 132, 3263-3272].

Since we know so little about mysticete hearing, it is difficult to be confident in any such prediction. However there are a few important concerns that should be highlighted and discussed with regard to their model and its predictions.

One major concern is that their model is not constructed at the organismal level, but instead examines a portion of the hearing apparatus, the tympanoperiotic complex, with the primary focus on the ossicular chain. This concern points to another deficiency in the model that Tubelli and his colleagues have offered. They do not provide any evidence that a validation test has been performed, and this warrants caution in accepting their results and conclusions. Validation may be particularly difficult to

accomplish with a model that only considers a small part of an organism. Nevertheless, validation is an important step that must completed before we can confidently accept their results.

A transfer function expresses the transformation of input into output. Both input and output are normally defined for specific locations. The output location for the middle ear is relatively well-defined: the stapes footplate, or the juxtaposed cochlear vestibule, just inside the oval window. The input location or locations also need to be defined so that discussing the "middle ear transfer function" (METF) will have some salience. And here lies another problem, Tubelli and his colleagues propose to formulate an anatomically accurate finite element model of the minke whale middle ear so that the METF may be constructed. But knowing the anatomy accurately is not sufficient. The mechanical effects of the incident sound on the anatomy must also be known. Tubelli et al. have, more or less, arbitrarily chosen two specific places for the mechanical input, and consequently obtain two different transfer functions. If they were to pick some other input location (or refine or enlarge or combine some input locations), a different METF would result. So, to be accurate, Tubelli et al. must admit that they have ***not*** constructed "***the*** METF for the minke whale", but instead have constructed two possible transfer functions out of many such transfer functions, some relevant and some irrelevant.

So, it is reasonable to ask, how should the METF be constructed? It is also reasonable to argue that what is needed is an acoustic model of the entire head of the animal (as we have shown in the RESULTS section), since it is the head that is exposed to acoustic waves from the environment. Such an acoustic model of the entire head allows for the determination of the acoustic input to the middle ear, without the need for any predetermined bias or arbitrary selection. The *systemic* (whole head) approach that we use to address the question of input location is also more likely to reveal factors and mechanism that may be unstudied, unknown, or as yet undiscovered. As a consequence, a case can be made that the systemic approach is also the most effective means for understanding all possible inputs that cause motion of the middle ear. Only to an uncertain and possibly small degree can such information be obtained by observation of only a small portion of the anatomy.

Another matter of concern is the control of the error with which the transfer function is computed. The Tubelli et al. (2012) finite element model of the middle ear is not described completely. For instance it is not clear whether the elements are linear or quadratic or cubic. This may have significant implications for the accuracy of their model. Furthermore, we must also note that the authors apparently did not test their model for convergence, in other words they have not quantified the error in their discrete model. Is their model expected to be 10% in error or 50% in error? We do not know because the authors either have not run these standard tests on their model or have published an incomplete report.

With the exception of their model's input location(s) (as noted above), the assumptions that went into the construction of the Tubelli model are technically justifiable. However, the authors go significantly awry in the parametric study of the damping portion of their model. Specifically, Tubelli and his colleagues assume Rayleigh stiffness-proportional damping with a constant of 10^{-5} seconds. This corresponds to roughly 3% of critical damping for a 1.0 kHz frequency or 30% of critical damping for a 10 kHz frequency. Correspondingly, if the authors undertake to investigate, by a parametric study, the Rayleigh stiffness-proportional damping with a constant of 10^{-3} seconds, then they in fact propose to study the middle ear as a strongly super-critically damped system. This amount of damping is approximately three times the critical damping for one kilohertz and 30 times the critical damping for 10 kHz. This amount of damping is unreasonable, and it is incorrect for them to claim that their study shows that the Rayleigh damping coefficient is one of the most important parameters in their model.

The realistic range for the Rayleigh stiffness-proportional damping coefficient may be 1×10^{-5} seconds to 5×10^{-5} seconds, and the corresponding change in their transfer functions would be much less dramatic. The damping coefficient is not likely to be a significant factor in a reasonably accurate model.

RELATED PROJECTS

Our current project, to CT scan an entire baleen whale and build a vibroacoustic model of it, is an outgrowth of an effort that was originally supported as a pilot project in 2004 by Dr. Frank Stone at the Chief of Naval Operations Environmental Readiness Division. That innovative project resulted in the development of the *vibroacoustic toolkit* (VTk) and a number of published papers (Krysl *et al.*, 2006; Cranford *et al.*, 2007; McKenna *et al.*, 2007; Cranford *et al.*, 2008a; Cranford *et al.*, 2008b; Krysl *et al.*, 2008; Cranford *et al.*, 2010; McKenna *et al.*, 2011; Barroso *et al.*, 2012; Castellazzi *et al.*, 2012; Cranford and Krysl, 2012; Krysl *et al.*, 2012a; Krysl *et al.*, 2012b; Ary *et al.*, 2014; Krysl and Cranford, 2014; Oberrecht *et al.*, 2014). That initial success has led directly to an ongoing project to synthesize odontocete audiograms based upon the CT scanning and vibroacoustic modeling methodology we developed. We recently passed two significant milestones, (1) validating the vibroacoustic modeling methodology by simulating sound production and beam formation in the bottlenose dolphin and matching it to previously published results with live dolphins (Cranford *et al.*, **2014**), and (2) producing synthetic audiograms for a mysticete whale and identifying the mechanism that allows them to have increased sensitivity to low frequency sounds.

A triple faceted project, *Virtual Experiments in Marine Bioacoustics: Whales, Fish, and Anthropogenic Sound*, was supported by an award from the Office of Naval Reseach, (N00014-09-1-0611). The **first** part of that project investigated whether our numerical vibroacoustic methodology could be applied to a completely different group of marine organisms, fish. Experts have long puzzled over how fish discriminate the frequency and direction of progressive, relatively long wavelength sounds. Our research suggests that the three pairs of otholiths within their hearing apparatus actually “rock” in response to such sounds (Krysl *et al.*, 2012a; Schilt *et al.*, 2012). Rocking otoliths have important implications for our understanding of fish hearing, challenging long-standing traditional ideas. The **second** part of this multifaceted project includes an effort to build a portable device to measure elasticity and sound speed in excised tissue samples in a laboratory setting. A prototype has been built and is being tested (Oberrecht *et al.*, 2014). The **third** and final aspect of this project proposes to validate our vibroacoustic models by comparing the simulation results to the psychoacoustic hearing experiments with live dolpins. The hearing experiments with the live dolphin were completed at the University of Hawaii. We are currently building a vibroacoustic model for a bottlenose dolphin so that we can run the corresponding numerical simulations.

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